Comparison of Serum Biochemical Profiles of Male Broilers with Female Broilers and White Leghorn Chickens

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ABSTRACT

Male broiler chickens were compared to female broiler chickens and male White Leghorns with respect to a 15-parameter serum biochemical profile at 9, 20, 30 and 42 days of age in order to determine which, if any, of the parameters tested might be useful in the identification of birds susceptible to sudden death syndrome. In comparison to female broilers, male broilers had significantly lower ($p < 0.01$) total protein levels at 20 days of age and significantly higher ($p < 0.01$) cholesterol levels at 30 days of age. Compared to male White Leghorns, in which sudden death syndrome has not been reported, male broilers had significantly lower ($p < 0.01$) levels of cholesterol and creatinine at nine days of age, total protein at 9 and 20 days of age and albumin at 20 days of age and significantly higher ($p < 0.01$) levels of potassium at nine days, uric acid at 9, 20 and 30 days, lactate dehydrogenase at 20, 30 and 42 days and cholesterol at 30 days of age.

RÉSUMÉ

Cette expérience visait à comparer des poulets de gril mâles à des poulets de gril femelles et à des Leghorn blancs mâles, au moyen d'un profil biochimique sérique de 15 paramètres, à l'âge de neuf, 20, 30 et 42 jours, afin de déterminer si l'un ou l'autre des paramètres pourrait s'avérer utile pour identifier les sujets susceptibles au syndrome de mort subite. Comparativement aux poulets de gril femelles, les poulets de gril mâles affichèrent un taux de protéines sériques significativement plus bas ($p < 0,01$), à l'âge de 20 jours, mais un taux de cholestérol significativement plus élevé ($p < 0,01$), à l'âge de 30 jours. Par rapport aux Leghorn blancs mâles, chez qui on n'a pas encore diagnostiqué le syndrome de mort subite, les poulets de gril mâles affichèrent des taux significativement plus bas ($p < 0,01$) de cholestérol et de créatinine, à l'âge de neuf jours, de protéines totales, à l'âge de neuf et 20 jours, et d'albumine, à l'âge de 20 jours. Ils affichèrent cependant des titres significativement plus élevés ($p < 0,01$) de potassium, à l'âge de neuf jours, d'acide urique, à l'âge de neuf, 20 et 30 jours, de lactate-déshydrogénase, à l'âge de 20, 30 et 42 jours, ainsi que de cholestérol, à l'âge de 30 jours.

INTRODUCTION

Serum biochemical profiling has been used in several species of domestic livestock to monitor herd health and to detect subclinical disease. Application of this technique to commercially raised poultry flocks has been limited by a lack of suitable reference ranges for most of the parameters being tested although much work has been done on specific individual parameters. In addition, much of the information that is available is based on small sample numbers, limited parameters and often outdated analytical techniques. Nonspecific "avian" values are not adequate because hematological and biochemical status is a reflection of many factors such as sex, age, breed, diet, management and stress level (1-3).

Altman and Dittmer (4) and Mitruka and Rawnsley (5) reported normal hematological and biochemical reference values for male and female adult White Leghorns. Ross *et al* (6) published "comparison" values for 12 plasma biochemistry parameters for healthy six week old broilers from 21 flocks of three producers. In some cases the trends and correlations noted in their paper are not substantiated in later work (2). However, in support of the use of biochemical profiling for detection of subclinical disease, the authors noted that a few months after sampling an outbreak of fatty liver and kidney syndrome occurred in the flocks of one of the three producers. In these same flocks it had been noted that cholesterol and glucose values were lower than values obtained in the other flocks and it was suggested that this finding reflected the presence of subclinical disease. The effect of age (during the first six weeks) on the biochemical profile of young, growing broilers, has not been adequately assessed although Woodard *et al* (7) compared several hematological parameters of partridges at one and seven years of age, and Ross *et al* (2) reported several parameters in young broilers at intervals during the first eight weeks.

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Sudden death syndrome (SDS) is a fatal disease specific to otherwise healthy, fast-growing broiler chickens. Sudden death syndrome can occur as early as three days of age and mortality continues throughout the growing period. It is the primary cause of broiler mortality in Canada and represents substantial economic loss to the producer (8-11). Death occurs suddenly with a short wing-beating convulsion and the specific cause is not known. Susceptible broilers are indistinguishable from their healthy penmates prior to death (11).

The primary objective of this experiment was to compare the 15 biochemical parameters used in a standard serum profile of healthy male broiler chickens (MB) with those of female broilers (FB) and male White Leghorns (MWL), in an attempt to determine differences which would suggest metabolic events predisposing MB to SDS or which might be used to select MB which are more prone to SDS. The FB have a very low incidence of SDS (9,11) and SDS has never been reported in MWL, therefore these groups were compared to MB. An effective screening procedure would allow for the establishment of SDS resistant stock through selective breeding and also prevent loss in susceptible flocks through preclinical monitoring.

TABLE I. Commercial Broiler Ration (Crumble)

	Starter	Grower-finisher
Feeding period	0-21 days	21-42 days
Protein (%)[a]	22	20
Energy (kcal ME/kg)[a]	3075	3150
Feed Ingredients		
Corn	50.00%	58.75%
Soyabean meal	40.15	31.00
Animal-vegetable blend fat	5.50	6.00
Calcium phosphate	2.00	2.00
Limestone	1.25	1.20
Iodine salt	0.25	0.25
Vitamin-mineral mix[b]	0.50	0.50
DL-methionine	0.09	0.007
Coban (monensin sodium)	0.04	0.04

[a]Calculated values.

[b]Vitamin-mineral mix (per kilogram of diet): vitamin A (10,000 IU/g), 8000 IU; vitamin D_3 (200,000 IU/g), 1600 IU; vitamin E (20,000 IU/g), 11 mg; riboflavin (53 mg/g), 9 mg; α calcium pantothenate (80 g/lb), 11 mg; vitamin B_{12} (60 mg/g), 13 mg; niacin, 26 mg; choline chloride (50%) (74% choline), 900 mg; vitamin K (Hetrazeen 35.27 mg/g), 1.5 mg; folic acid (13.23 mg/g), 1.5 mg; biotin (2%), 0.2 mg; santoquin (25%), 125 mg; manganous oxide (56% Mn), 55 mg; selenium premix (200 mg/kg), 0.1 mg; zinc oxide (80% Zn), 50 mg; copper sulfate (25% Cu), 5 mg; ferrous oxide (69.94%) Fe), 30 mg

MATERIALS AND METHODS

CHICKENS

Two hundred commercially obtained MB, (Hubbard x Hubbard), 200 FB (Hubbard x Hubbard) and 200 MWL (Shaver) day-old chicks were each divided into four groups of 50 and the groups were then housed in individual floor pens at Arkell Poultry Research Station, University of Guelph. A commercial corn-soy broiler ration (Table I) was fed *ad libitum* to all pens and water was available at all times. The lighting schedule consisted of 23 h of light and 1 h of dark for the duration of the experiment.

EXPERIMENTAL DESIGN

Blood samples were collected by cardiac puncture at 9, 20, 30 and 42 days of age from five birds per pen (a total of 20 birds per sex or breed). Birds from one pen at a time were chosen and bleeding took place between 0900 and 1030 h. Once bled, birds were not resampled at subsequent sampling times. Feed was not removed prior to sampling. The blood was allowed to clot and serum was removed within 2 h of collection. A standard biochemical profile was determined for each sample using an automated Parallel® analyzer (American Monitor Corporation, Mississauga, Ontario). All values were determined using American Monitor reagent systems.

The profile consisted of calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chloride (Cl), glucose, cholesterol, total protein, albumin, creatinine, total bilirubin, uric acid (UA), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST). The level of calcium was determined based upon a color product formed when calcium reacts with o-cresolphthalein complexone in an alkaline medium. Phosphorus assay depends on formation of phosphomolybdenum blue. The level of magnesium was measured spectrophotometrically using the metallochrome dye Calmagite to form a red-colored complex. Sodium and potassium were determined by flame photometry. Chloride was determined using the indicator 2,4,6-tripyridyl-S-triazine. Glucose was determined by the glucose oxidase reaction and cholesterol was determined enzymatically and included the free and esterified forms. The total protein and albumin levels were determined with biuret and bromocresol green reagents respectively. The creatinine assay utilized alkaline picrate. The total bilirubin determination was based upon diazotization. Uric acid levels were determined based on activated oxidation of uric acid coupled to the reduction of phosphotungstate to a colored product. The activity of serum LDH was determined kinetically by the rate of formation of a blue chromophore complex. Serum AST activity correlates to the disappearance of NADH in an enzymatic reaction.

STATISTICAL ANALYSIS

Data were analyzed by multivariate analysis of variance using the General Linear Models procedure (SAS Institute Inc., North Carolina). A mean value for each parameter in each group at each age was determined based upon a sample of 20 birds per strain. Data obtained for MB were directly compared to those of FB and MWL at each of the four ages with probability level $p < 0.01$ using type III mean squares determination.

RESULTS

The mean and standard deviation for each parameter of 9, 20, 30 and 42 days of age for the three types of chicken are presented in Table II. There were fewer significant differences between the sexes than there were between MB and MWL. A strain-age interaction existed for total protein and cholesterol at $p < 0.01$, so comparisons were made only between the types of chickens at 9, 20, 30 and 42 days of age. Age effect was not considered here. The significant differences ($p < 0.01$) between MB

TABLE II. Biochemical Values Obtained for Male Broilers, Female Broilers and Male Leghorns at 9, 20, 30 and 42 Days

			Parameter													
Type of Chicken	Age (days)	n	Calcium (mmol/L) (x4= mg/dL)	Phosphorus (mmol/L) (x3.1= mg/dL)	Magnesium (mmol/L) (x2.4= mg/dL)	Sodium (mmol/L) (x1= mEq/L)	Potassium (mmol/L) (x1= mEq/L)	Chloride (mmol/L) (x1= mEq/L)	Glucose (mmol/L) (x18= mEq/L)	Cholesterol (mmol/L) (x38.7= mEq/L)	Tot Prot G/L (x0.1= mg/dL)	Albumin G/L (x0.1= mg/dL)	Creatinine µmol/L (x0.011= mg/dL)	T Bili µmol/L (x0.058= mg/dL)	Uric Acid µmol/L (x0.017= mg/dL)	LDH U/L	AST U/L
MB	9	20	2.59 ± 0.09[a]	2.81 ± 0.35	1.49 ± 0.15	145 ± 2.9	6.33 ± 0.89	107 ± 1.6	14.9 ± 1.2	3.44 ± 0.51	25.6 ± 1.7	11.9 ± 4.0	40.7 ± 3.5	3.10 ± 0.64	639 ± 175	801 ± 153	273 ± 133
	20	19	2.63 ± 0.15	2.88 ± 0.33	1.01 ± 0.17	148 ± 2.5	5.97 ± 0.98	110 ± 1.8	15.7 ± 1.2	2.98 ± 0.37	26.5 ± 1.9	11.8 ± 1.0	40.2 ± 2.9	3.05 ± 0.40	625 ± 180	869 ± 158	190 ± 40
	30	20	2.73 ± 0.08	2.93 ± 0.26	1.09 ± 0.11	154 ± 2.3	4.84 ± 0.75	106 ± 3.6	15.7 ± 1.1	4.23 ± 0.39	34.4 ± 2.7	12.9 ± 1.2	44.8 ± 5.6	3.11 ± 1.3	374 ± 92	817 ± 259	184 ± 38
	42	20	2.73 ± 0.13	2.58 ± 0.23	1.03 ± 0.12	150 ± 2.8	4.74 ± 0.84	112 ± 1.5	15.1 ± 1.3	3.23 ± 0.31	32.6 ± 2.3	13.7 ± 1.4	52.3 ± 5.7	2.60 ± 0.50	380 ± 77	966 ± 934	254 ± 293
FB	9	20	2.56 ± 0.20	2.75 ± 0.30	1.47 ± 0.13	147 ± 3.6	6.23 ± 1.1	110 ± 2.0	14.2 ± 0.93	3.33 ± 0.36	26.8 ± 1.3	11.8 ± 0.08	40.1 ± 9.0	3.15 ± 0.37	607 ± 135	886 ± 93	348 ± 125
	20	20	2.69 ± 0.09	2.76 ± 0.18	0.99 ± 0.14	148 ± 2.6	5.86 ± 0.70	111 ± 2.3	14.4 ± 0.76[b]	3.12 ± 0.29	28.8 ± 1.8[c]	12.9 ± 0.08[c]	46.3 ± 2.1	3.15 ± 0.49	627 ± 136	852 ± 206	180 ± 22
	30	20	2.70 ± 0.10	3.11 ± 0.16	1.12 ± 0.10	155 ± 1.5	5.08 ± 0.70	108 ± 5.3	14.9 ± 1.1	3.72 ± 0.50[c]	34.3 ± 2.9	13.0 ± 1.2	46.9 ± 4.3	2.70 ± 0.57	341 ± 78	876 ± 270	182 ± 18
	42	20	2.76 ± 0.10	2.63 ± 0.21	1.00 ± 0.08	151 ± 2.7	4.83 ± 0.79	112 ± 2.1	14.7 ± 0.75	3.13 ± 0.39	34.2 ± 1.8	14.2 ± 0.08	52.1 ± 6.7	2.40 ± 0.75	388 ± 70	731 ± 304[b]	199 ± 21
MWL	9	20	2.75 ± 0.16[b]	2.78 ± 0.26	1.41 ± 0.12	144 ± 4.6	5.00 ± 1.2[c]	108 ± 2.2	14.8 ± 1.2	4.03 ± 0.43[c]	30.8 ± 1.1[c]	13.4 ± 1.0[b]	49.1 ± 7.0[c]	3.14 ± 0.59	374 ± 82[c]	651 ± 167	251 ± 117
	20	17	2.70 ± 0.11	2.81 ± 0.23	1.04 ± 0.17	145 ± 2.9	5.78 ± 0.76	110 ± 1.9	16.7 ± 0.97[b]	3.28 ± 0.20[b]	30.6 ± 1.8[c]	14.0 ± 0.09[c]	45.0 ± 2.7	2.80 ± 0.62	466 ± 60[c]	630 ± 162[c]	196 ± 35
	30	20	2.64 ± 0.13	2.82 ± 0.16	1.01 ± 0.06	155 ± 1.5	4.20 ± 0.44	108 ± 4.0	16.4 ± 0.90	3.25 ± 0.44[c]	33.9 ± 2.2	13.3 ± 1.6	48.8 ± 4.5	2.35 ± 0.75[b]	245 ± 49[c]	490 ± 138[c]	188 ± 16
	42	20	2.73 ± 0.25	2.47 ± 0.34	0.95 ± 0.06	148 ± 2.2	4.41 ± 0.59	109 ± 2.5	15.5 ± 1.0	2.93 ± 0.51	32.9 ± 1.7	13.9 ± 1.9	50.5 ± 8.6	2.55 ± 0.92	325 ± 61	358 ± 116[c]	183 ± 12

[a]Mean ± standard deviation

[b,c]Significantly different from MB for specified parameter at specified age (b = $p < 0.05$, c = $p < 0.01$)

MB - Male broiler chickens; FB - Female broiler chickens; MWL - Male White Leghorn chickens

and FB were increased levels of cholesterol in MB at 30 days of age and increased levels of total protein and albumin in FB at 20 days of age.

The differences between the MB and MWL were more extensive. The parameters significantly higher ($p < 0.01$) in MB were potassium at nine days, uric acid at 9, 20 and 30 days, LDH at 20, 30 and 42 days, and cholesterol at 30 days of age. The parameters found to be significantly higher in MWL were cholesterol and creatinine at nine days and total protein and albumin at 9 and 20 days of age. No significant differences were observed in phosphorus, magnesium, sodium, chloride, glucose, total bilirubin and AST.

The distribution and causes of mortality are outlined in Table III. There was 4% (8/200) mortality due to SDS in the MB and 0.5% (1/200) in FB. Sudden death syndrome was not diagnosed in MWL.

DISCUSSION

There are major differences in many serum parameters of MB and MWL of similar age and diet and fewer differences between MB and FB (Table II). Breed and strain of chicken, laboratory procedures and methods of statistical analysis should also be considered when published reference data are to be used for comparison.

Ross *et al* (2) reported significantly higher levels of total protein, albumin, uric acid and potassium in MB with higher calcium in MWL. The higher level of uric acid in their broilers was attributed to kidney damage as a result of infectious bronchitis (IB) vaccination. However, uric acid levels can show great variability between species (12) and will vary directly with dietary protein level, total food intake and bodily requirements for amino acids (13). Laying birds generally have lower levels of uric acid than nonlaying birds (2,14) and our results support this finding. The level of uric acid in all strains rose between age 9 and 20 days but dropped markedly with the change from 22% protein starter diet to a 20% protein grower-finisher diet at 21 days of age. Serum magnesium levels also vary directly with dietary intake and have no effect on the incidence of SDS (10).

TABLE III. Mortality Distribution

Type of Chicken	Age	SDS	Other[a]	Total
MB	< 9 days	0	2[b]	2
	10-20	4	0	4
	21-30	1	0	1
	31-42	3	2[c,d]	5
	Total (%)	8/200 (4%)	4/200 (2%)	12/200 (6%)
FB	< 9 days	0	4[b]	4
	10-20	0	2[d]	2
	21-30	0	0	0
	31-42	1	2[c,f]	3
	Total (%)	1/200 (0.5%)	8/200 (4%)	9/200 (4.5%)
MWL	< 9 days	0	1[f]	1
	10-20	0	0	0
	21-30	0	0	0
	31-42	0	0	0
	Total (%)	0/200 (0%)	1/200 (0.5%)	1/200 (0.5%)

[a]Other causes of mortality were:
[b]starve-out/dehydration, [c]ascites, [d]bleeding trauma
[e]culled for lameness, [f]no diagnosis made
MB - Male broiler chicken; FB - Female broilder chicken;
MWL - Male White Leghorn chicken

In this study serum cholesterol levels were significantly ($p < 0.01$) higher in MWL than MB at nine days of age but were significantly higher in MB than in FB or MWL at 30 days of age. Ross *et al* (2) reported higher levels of cholesterol in male Brown Leghorns than in females at 16 weeks of age. Cholesterol was also higher in six week old male Brown Leghorns than in MB although the 95% confidence limits were similar. Riddell and Orr (8) reported that total lipid levels in control broilers dying from SDS were similar except for elevated levels in two SDS cases. Rotter *et al* (17) found no effect of source of dietary fat on blood lipid parameters (total lipid, triglyceride and cholesterol) in broiler chickens at four and seven weeks of age. The four and seven week cholesterol levels were 2.84 and 3.57 mmol/L (110 and 138 mg/dL) and were similar to those reported here (2.98, 4.23 and 3.23 mmol/L at 20, 30 and 42 days of age respectively).

The fowl lacks creatine-dehydrating mechanisms and therefore has very low serum levels of creatinine and higher circulating levels of creatine (14). The importance of the significantly higher level of creatinine which was noted in nine day old MWL in this trial is not known. Other researchers have used creatinine as an indicator of muscle metabolism but this remains to be confirmed.

Total serum protein is influenced by breed, age, physiological state, environment and antigen exposure and levels can be extremely variable (16). Serum albumin will increase when protein intake exceeds the amount required for growth and maintenance. In addition 50% of the calcium present in blood is bound to albumin (16) and may explain the higher concurrent total protein, albumin and calcium levels in MWL at 9 and 20 days of age. Ross *et al* (2) found increased serum calcium to be a characteristic of young layer type birds.

In this study potassium was higher for MB at nine days of age. Ross *et al* (6) found MB to have significantly higher levels of potassium than previously reported values of WL (unspecified sex) at six weeks of age.

Riddell and Orr (8) determined serum electrolyte levels in broilers dying from SDS and in control broilers 12-21 days of age bled at intervals after death. A rapid postmortem elevation in serum potassium was found in control and SDS birds emphasizing the importance of obtaining a blood sample from a bird dying of SDS as rapidly after death as possible if the involvement of potassium as an etiology is to be investigated.

In this study LDH was significantly higher in MB than in MWL at 20, 30 and 42 days of age. Since skeletal and cardiac muscle can be significant sources of LDH in serum this may reflect the larger muscle mass and histologically evident focal myofibrillar degeneration commonly present in clinically normal broilers (8) or an increased cardiac workload in the heavier broilers. Bell (15) reported higher levels of LDH in young growing birds with no difference between male and female.

Some serum biochemical parameters in growing broiler chickens are quite different from adult levels (5,6) with calcium, cholesterol, albumin and total protein being lower in the younger birds. Ross *et al* (2) found that over the first eight weeks of age in broiler chickens, there was an increase in total protein, globulin and glucose and a decrease in potassium and phosphorus. Woodard *et al* (7) compared one year old and seven year old partridges and found that serum glucose, AST, potassium, cholesterol, uric acid and albumin increased significantly with age.

In this trial serum calcium, sodium, creatinine, total protein and albumin levels increased over the six week period while magnesium, potassium, total bilirubin and AST decreased although statistical analysis of these trends was not the objective of this paper.

No consistent biochemical differences that might indicate subclinical SDS were noted for MB (high SDS incidence) when compared to FB and MWL (low and no SDS incidence respectively). The higher levels of LDH and potassium in MB could be a reflection of their larger muscle mass or may reflect heart or skeletal muscle damage. Further work is required to determine whether these findings are indicative of a predisposition to SDS.

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